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DETERMINATION OF CITRIC ACID.

By DAVID S. PRATT, *Assistant Chemist, Division of Foods.*

HISTORICAL NOTE.

At the present time there is no practical method for the determination of citric acid in the presence of other fruit acids. In the absence of other organic acids, barium citrate may be precipitated in a 50 per cent alcoholic solution. This precipitate may be filtered, dried, and weighed directly[1] or may be converted into barium sulphate and weighed as such.[2] When citric acid is accompanied by malic, tartaric, or some other similar acid, however, the procedure is very complicated and unsatisfactory. These other acids are at least partially precipitated with the citric, although, as Jörgensen[3] points out, it is possible to effect a partial separation by precipitating the barium salts in 26 per cent alcohol. In this solution barium citrate is nearly insoluble and barium malate and tartrate rather soluble. Unfortunately the separation is very far from sharp, and the necessity of repeated reprecipitation makes the determination tedious and unsatisfactory. Methods based upon the evolution of carbon monoxid from citric acid when heated with concentrated sulphuric acid at 100° C.[4] and upon precipitation of the citric acid by means of quinin[5] are subject to the same limitations.

It has long been known that oxidizing agents in mineral acid solution break down citric acid with the production of acetone and carbon dioxid. Péan de Saint Gilles[6] notes that potassium permanganate and sulphuric acid effect this change at temperatures above 80° C. Fleischer[7] obtained no acetone, however, in the absence of mineral acid, and Phipson[8] states that a solution of free citric acid at ordinary temperatures gives, with potassium permanganate, oxalic acid but no acetone. The work of these early writers was carefully gone over by Wöhlk,[1] who found that the formation of oxalic acid is due only to secondary oxidation or to an excess of potassium permanganate, and that the first action of potassium permanganate on citric acid in acid solution is the formation of acetone dicarbonic acid in accordance with the following equation:

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[1] Bacon and Dunbar. U. S. Dept. Agr., Bureau of Chemistry Cir. 78, p. 8.

[2] Creuse. Jahresber. Chem., 1873, p. 970.

[3] Zts. Nahr. Genussm., 1907, 13 : 241; 1909, 17 : 396.

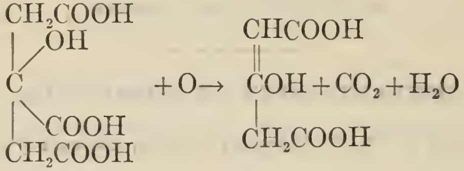
[4] Spica. Chem. Ztg., 1910, 34 : 1141.

[5] Sindet. Bull. soc. chim. Par., 1896 (3), 15 : 1162.

[6] Jahresber. Chem., 1858, p. 585.

[7] Ber. d. chem. Ges., 1872, 5 : 353.

[8] J. Chem. Soc., 1862, 15 : 141.



The enol form is given, as the dicarboxylic acid reacts with acid ferric chlorid to give a reddish color [2] characteristic of hydroxyl, but the keto form also exists, as the substance gives a red color in alkaline solution with sodium nitroprussid.[3, 4] These reactions are offered by the authors quoted as qualitative tests for citric acid, but they require much time and careful manipulation.

Stahre's [5] test is comparatively simple. It consists in acidifying the solution to be tested with sulphuric acid, adding bromin water, and heating with potassium permanganate. A white turbidity results in the presence of citric acid. Wöhlk [1] has shown that here again the test depends upon the formation of acetone dicarbonic acid, and that this in the presence of bromin precipitates penta-brom-acetone as a white milky cloud.

The most delicate and satisfactory test for citric acid is that proposed by Denigès.[6] His reagent is made by dissolving 5 grams of mercuric oxid in 20 cc of concentrated sulphuric acid and diluting with water to 100 cc. When this reagent is added to a solution containing citric acid or a citrate, the solution warmed and a potassium permanganate solution slowly added, a white precipitate is formed, characteristic of citric acid. This is due to the formation of a complex between the reagent and the acetone dicarbonic acid resulting from the oxidation of citric acid. Malic, tartaric, and other ordinary fruit acids give no precipitate; oxalic gives a precipitate upon addition of the reagent, but by filtering and adding potassium permanganate to the clear filtrate the interference is avoided. This test is explained in detail by Yoder [7] and need not be elaborated upon here. It should be noted, however, that it often fails in the presence of much sugar. In this event the citric acid must be separated and freed from the interfering substances by precipitation as barium citrate in 50 per cent alcoholic solution, washed with 50 per cent alcohol, and liberated by sulphuric acid. To the clear filtrate so obtained the test is applied. The test solution should be diluted until a few drops of potassium permanganate solution will give a faint color in the cold. The pink liquid is then heated. In the presence of citric acid the color fades and is replaced by a white turbidity characteristic of citric acid. If this is not obtained add potassium permanganate drop by drop, waiting between each addition until a clear solution free from manganese dioxid is obtained.

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[1] Zts. anal. Chem., 1902, **41**: 77.

[2] Favrel. Ann. chim. anal., 1908, **13**: 177.

[3] Bernh. Merk. Pharm. Ztg., 1903, **48**: 894.

[4] Spica. Gaz. chim. ital., 1901, **31**(2): 61.

[5] Nordisk. Farm. Tidsskrift, 1895, p. 141; Zts. anal. chem., 1897, **36**: **195**.

[6] Ann. chim. phys., 1899, **18**: 415.

[7] J. Ind. Eng. Chem., 1911, **3**: 644.

It has been suggested by Beau[1] that this method might be made quantitative for citric acid in milk. His idea was to mix the milk with Denigès's reagent, heat nearly to boiling and add drop by drop a 1 per cent potassium permanganate solution. A slight excess, later destroyed by hydrogen peroxid, was used, the precipitate dried and weighed. Theoretically this method is faulty, it being impossible completely to oxidize citric acid to acetone dicarbonic acid without at the same time carrying the reaction further. In fact, Wöhlk[2] found that acetone dicarbonic acid alone breaks down in aqueous solution at 0° C., giving acetone and carbon dioxid. It is evident, therefore, that no method based upon the partial oxidation of citric acid can be quantitative.

This suggested, however, the final attack on the problem. If citric acid could be quantitatively oxidized to acetone, this might be easily removed from the other products of the reaction by distillation and determined. From the results of Péan, Fleischer, and Wöhlk it appeared that the oxidation should be carried on in acid solution in order to give acetone as the final product. It is evident that all acetone dicarbonic acid formed will break down at the temperature of distillation, giving acetone.

EXPERIMENTAL WORK.

A number of oxidizing agents were tried on known amounts of citric acid in solutions acidified with various mineral acids. Of all the combinations experimented with, a solution of potassium permanganate acting in the presence of sirupy phosphoric acid was most satisfactory. Sulphuric acid under some conditions is partially reduced to sulphuric dioxid, and this gives a precipitate with Denigès's reagent. Hydrochloric acid must be avoided, as it dissolves the final precipitate. Potassium bichromate decomposes citric acid in acid solution, yielding acetone, but is less satisfactory than permanganate.

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[1] Rev. gén. du lait, 1904, p 386.

[2] Loc. cit.

The great advantage possessed by phosphoric acid over sulphuric acid will be touched upon later. With certain precautions described under details for carrying out the method, a solution containing 0.5 gram of potassium permanganate per liter gave very good results.

At this point it became necessary to decide upon the best method for determining acetone in the distillate. The ordinary methods depending on iodin solution are not well suited to this case, inasmuch as other volatile bodies are generally present that are also oxidized by an alkaline iodin solution. A very satisfactory gravimetric method was introduced by Denigès.[1] His mercuric sulphate reagent described above forms with acetone an insoluble white precipitate similar in appearance to that formed with acetone dicarbonic acid. The method as given is to heat a very dilute acetone solution, containing not over 1 gram of acetone per liter, with the reagent in excess in a sealed flask immersed in boiling water. The precipitate is dried at 100° C., and its weight multiplied by the factor 0.06 to obtain the corresponding amount of acetone. To the precipitate so obtained, Denigès gives the formula $(2HgSO_4.3HgO)_3.4CH_3COCH_3$, with a theoretical factor of 0.0584. He further states that long-continued heating at 110° C. causes the loss of acetone, giving the compound $2HgSO_4.3HgO.CH_3COCH_3$.

However, as Oppenheimer[2] points out, no analyses of the product dried at 100° C. and utilized in the determination are given. In fact, Oppenheimer arrives at a different composition and an acetone factor of 0.052. He states that the compound does not lose acetone at 110° C., but hesitates to state definitely its formula. From these results it is evident that an empirical factor must be used to convert the weight of precipitate into terms of citric acid. The following table gives the determinations used in arriving at this factor and the calculated amount of citric acid when using it. The method employed in obtaining these results is explained in detail later:

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[1] Ann. chim. phys., 1899, **18**: 400; Compt. rend., 1898, **127**: 963; 1898, **126**: 1868.

[2] Ber. d. chem. Ges., 1899, **32**: 986.

Determination of factor.

Amount of citric acid used.	Weight of precipitate.	Calculated factor.	Citric acid found by using factor 0.220.
Gram.	*Gram.*		*Gram.*
0.020	0.104	0.187	0.023
.049	.234	.208	.051
.052	.227	.224	.050
.080	.343	.231	.075
.097	.453	.214	.100
.104	.430	.240	.094
.104	.451	.229	.099
.104	.445	.232	.098
.104	.442	.234	.097
.107	.510	.210	.112
.128	.559	.229	.112
.128	.556	.230	.122
.146	.679	.215	.149
.246	1.181	.208	.259
.491	2.364	.208	.520
.046	.213	.216	.049
.049	.220	.221	.048
.050	.231	.216	.051
.049	.205	.239	.045
.050	.211	.237	.046
.096	.468	.205	.103
.096	.437	.219	.096
.049	.208	.235	.046
.049	.225	.218	.050
Average.	....	.220	

THE METHOD.

The apparatus needed is very simple and consists of a 500 cc distilling flask connected with a condenser, preferably of the spiral type. A small dropping funnel is inserted in the neck of the flask, protruding about half an inch through the stopper and drawn down to the dimensions of an ordinary medicine dropper. A longer tube is apt to form bubbles of steam which force the remainder of the potassium permanganate rapidly into the flask and thus interfere with its regular addition, which should be drop by drop. This allows the acetone formed in the reaction to be distilled off as fast as formed, thus preventing any loss by oxidation. To make this doubly sure, some device should be included in the flask to cause steady ebullition. For this purpose nothing is better than a glass rod with half an inch of small tubing sealed on the lower end. It is essential that the small hollow end be filled with air when the heating of the liquid is commenced. After cooling this cavity fills with liquid, which should be shaken out before using. The device is not new, but it does not receive the recognition it merits in many cases where regular boiling without bumping is desired. The distillate is received in a liter Erlenmeyer flask containing 30 to 40 cc of Denigès's reagent.[1] This is made as follows: Add about 500 cc of water to 50 grams of mercuric oxid;

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[1] Loc. cit.

then add 200 cc of concentrated sulphuric acid with constant stirring, and heat the mixture, if necessary, on a steam bath until the solution is complete. After cooling, make up to a liter and filter.

The method of analysis is as follows: If a fruit juice is under examination, weigh 50 grams into a beaker and add 110 cc of 95 per cent alcohol to throw out the pectin bodies. After standing 15 minutes remove these by filtration[1] and wash the residue with 95 per cent alcohol in which citric acid is readily soluble. Dilute the filtrate with water to give approximately a 50 per cent alcohol content and add enough 20 per cent aqueous solution of barium acetate to precipitate the barium citrate completely. Stir, let stand until the precipitate partially settles, and filter. Wash the precipitate thoroughly with 50 per cent alcohol to remove the greater part of the sugar present. This may be done by filling the paper twice with the dilute alcohol. Place the filter paper and its contents in the beaker used for the precipitation and dry until all alcohol is removed. If desired, the same result may be accomplished by washing with ether before removing the filter paper from the funnel. Add about 50 cc of water and from 3 to 5 cc of sirupy phosphoric acid to the beaker containing the filter paper and precipitate. Upon warming, the barium citrate is completely dissolved. This is the chief reason for the use of phosphoric acid rather than sulphuric, which would throw out barium sulphate. Filter into a 100 cc measuring flask and make up to volume with washings from the filter paper in the beaker. This completely removes the barium citrate.

An aliquot, estimated to contain from 0.050 to 0.150 gram of citric acid, is measured into the distilling flask. About 5 to 10 cc of sirupy phosphoric acid, and 400 cc of hot water are added. When briskly boiling, add potassium permanganate solution (0.5 gram per liter), 1 to 2 drops per second, until a pink color throughout the solution shows the reaction to be complete. The distillate is received in a liter Erlenmeyer flask containing from 30 to 40 cc of Denigès's reagent just described. The distillation is continued until about 50 to 100 cc remain in the flask. The Erlenmeyer containing the distillate is then connected with a reflux condenser, and boiled gently for three-quarters of an hour after the solution turns milky. Filter hot through a Gooch crucible, wash with water, alcohol, and finally with ether, and dry in a water oven for half an hour. The weight of the precipitate multiplied by 0.22 gives citric acid.

With many solutions the precipitation of the barium citrate and its subsequent solution are unnecessary. In cases, however, where large amounts of sugar are present it is the safer procedure, and as the whole operation requires but little time and attention it is to be recommended. The presence of sugars seems to retard the oxidation of the citric acid, and moreover makes it difficult to determine when enough potassium permanganate has been added, as no definite end point can be reached.

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[1] A folded filter is satisfactory when but little citric acid is present, as in the case of fruits, and saves much time. The pectin bodies generally may be retained in the beaker and washed by decantation.

The precipitate obtained in this method is heavy and never causes any difficulty in filtering. It is a creamy white if properly obtained, and is very insoluble in hot or cold water, in alcohol, and in ether. The crucibles and flasks may be readily cleansed by using dilute hydrochloric acid in which the precipitate is easily soluble. Malic and tartaric acid do not interfere with the determination, as may be seen in the following table:

Determination of citric acid in the presence of tartaric and malic acids.

Citric acid used.	Malic acid present.	Tartaric acid present.	Weight of precipitate.	Citric acid recovered.
Gram.	*Gram.*	*Gram.*	*Gram.*	*Gram.*
0.050	0.000	0.010	0.230	0.051
.050	.000	.010	.235	.052
.050	.000	.096	.242	.053
.050	.046	.000	.230	.050
.050	.093	.000	.228	.050
.049	.057	.000	.245	.053
.052	.010	.000	.246	.054
.052	.047	.000	.230	.051
.053	.094	.000	.240	.053
.050	.048	.050	.215	.047
.050	.048	.050	.224	.049

The method is suited to the analysis of a great variety of substances besides fruits and fruit products. Among these might be mentioned citric acid in the presence of sulphuric acid or in crude calcium citrate, both occurring in the commercial manufacture of this acid: also various medicinal preparations, flavoring extracts, and any material containing citric acid. Duplicate results generally check to within 2 or 3 mg of citric acid.

[Cir. 88]

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